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Monitoring a mammalian nuclear membrane phase transition by intrinsic ultraweak light emission

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The first thermodynamic measurements of the intensity of light emission associated with native lipid peroxidation in a biological membrane are described. Kinetics of the radical chain reaction are shown to be sensitive to membrane structural phase and lipid dynamics. This is demonstrated by a novel measurement of a phase transition in the membrane of the intact mammalian nucleus. The apparent activation energies of lipid peroxidation in this system are also obtained for the first time. We suggest that this measurement may be more generally applicable as a method for monitoring membrane phase transitions.

Lipid peroxidation; Light emission; Nuclear membrane; Phase transition

1. INTRODUCTION

Lipid peroxidation, normally a weak radical chain reaction whose details remain largely unresolved [1], is of considerable interest due to experimental evidence of a relationship between its products and processes such as mutagenesis, carcinogenesis and aging [2,3].

We recently reported a native ultraweak light emission from isolated rat liver nuclei using a highly sensitive single photon counting instrument developed especially for the purpose of measuring the extremely weak optical fields known to emanate from most biological materials. The light emission was attributed to excited states produced in lipid peroxidation of the nuclear membrane [4].

Here we describe measurements of the temperature dependence of the light emission intensity. The measurements show that the kinetics of lipid peroxidation in the nuclear membrane are sensitive to lipid conformation, making possible the first observation of a phase transition in the membrane of the intact mammalian nucleus.

2. MATERIALS AND METHODS

Rat liver nuclei were isolated from male Wistar rats according to a procedure described by Windell and Tata [5] and suspended in 0.25 M sucrose medium containing 1 mM $MgCl_2$. The preparation was carried out at 3–4°C and light emission measurements were made immediately. The quality of the preparation was verified by phase contrast microscopy and the number of nuclei, determined by heamocytometry, was adjusted to 10^8 per 4 ml sample volume. Incubation conditions for ascorbate-driven lipid peroxidation were according to the methods described by Vaca et al. [6], i.e. 50 μM ascorbate and 10 μM $FeCl_2$.

Light emission measurements were made using a vacuum-isolated single photon counting apparatus constructed in this laboratory and described in [4]. Briefly, by isolation in vacuum, the ultimate capability of a selected photomultiplier tube (Hamamatsu R-375; wavelength response, 160–850 nm) is fully exploited by cooling the photocathode (5 cm diameter) to −45°C. In addition to the resulting low noise, vacuum isolation allows significant gain in sensitivity through reduced sample to photocathode distance. Photoelectron pulses from the detector are registered with a two-channel counter (Stanford Research Systems Inc., SR400) interfaced with a personal computer for data accumulation and analysis.

The sample of nuclei was maintained in a mini-incubation chamber with a fused silica window in close proximity to the photocathode. The mini-incubation chamber, equipped with ports for external introduction of liquid materials and gasses, encloses a steel Petri dish on a copper cooling and heating block. Sample temperature monitoring and control are accomplished using a thermistor-based thermometer capable of measuring temperature differences of 10^{-3}°C. Sample temperature was acquired and displayed simultaneously with photon counting data on the second channel of the counter mentioned above.

All measurements were made with unstirred samples, which were prepared as very thin layers of 3×10^8 intact nuclei sedimented under 2 mm of medium in 50 mm stainless steel Petri dishes (to avoid the long delayed delayed fluorescence of glass or plastic). A 4 ml suspension of 10^8 freshly isolated nuclei at 3–4°C was injected into the steel dish of the mini-incubation chamber maintained initially at the same temperature with an air flow of 10 ml/min. Photoelectron pulses were then counted during 10 s gate times as the temperature of the sample was increased to 37°C.

3. RESULTS AND DISCUSSION

The measurements were motivated by our assumption that the kinetics of the membrane-bound reactions could be sensitive to lipid dynamics. More generally, we suspected that these reactions, constrained to the 100 Å thickness of the membrane, itself structured on even smaller scales, might exhibit the non-classical kinetic

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characteristics of physically constrained systems [7]. Therefore, all measurements were made with unstirred samples. The rate of single photon detection from 3×10^8 nuclei ranged from approximately 16 to 610 counts/s.

Arrhenius plots of light emission intensity for both native and ascorbate-driven lipid peroxidation in intact nuclei are shown in Fig. 1. Emission intensity data in part (a) of the figure are comprised of 296 points obtained on heating a sample from 285K to 310K at a rate of 0.0084K/scc. The effects of fluctuations arising from the quantum detection process and source fluctuations are evident at lower count rates (and temperatures).

The Arrhenius plot of intensity of native emission from isolated nuclei (Fig. 1a) displays a discontinuity indicative of a phase transition. This figure also shows a least squares fit of the data from which we derive a transition temperature, T_c, of 28.509 ± 0.084°C. The observed transition temperature is consistent with those obtained by other methods in a variety of other membrane systems from various species, including for example *E. coli* raised at 36°C [8].

The apparent activation energies of the native radical chain reaction below and above T_c are 17.025 and 42.667 kcal/mol, respectively.

In a second experiment we found that the transition occurs at the same temperature (28.972 ± 0.084°C) in ascorbate-driven lipid peroxidation [6] (Fig. 1b), a system important in biochemical studies because the presence of ascorbate greatly enhances the rates of reaction. The activation energies observed in the catalyzed reaction were lower than those in native lipid peroxidation, as expected; 15.269 and 28.891 kcal/mol below and above T_c, respectively.

Interestingly, we observed that the transition is abolished in nuclei prepared in a frequently-used Tris buffer system, suggesting that Tris (buffer) significantly alters membrane properties (data not shown). This observation is consistent with freeze-fracture electron microscopy and NMR spectroscopy studies, which show both structural alteration and motional pinning of lipopolysaccharide when suspended in Tris buffer [9].

Although it remains to be confirmed in other systems, the ubiquity of membrane lipid peroxidation suggests that this new method may be of more general application. The approach differs in principle from the use of spin labels or fluorescent probes whose incorporation may alter membrane dynamics, relying instead on detection of the intrinsic light emission associated with lipid peroxidation. The method provides a well defined transition temperature, a general property of Arrhenius methods and, potentially, information about the dynamics of the transition in the vicinity of T_c; as seen in Fig. 1, the high resolution in temperature allows us to track the dynamical behavior continuously through the region of the transition, much as in a calorimetric technique [10]. Here though, the information contained in the data pertains to the relationship between the radical chain reaction kinetics and membrane molecular structure.

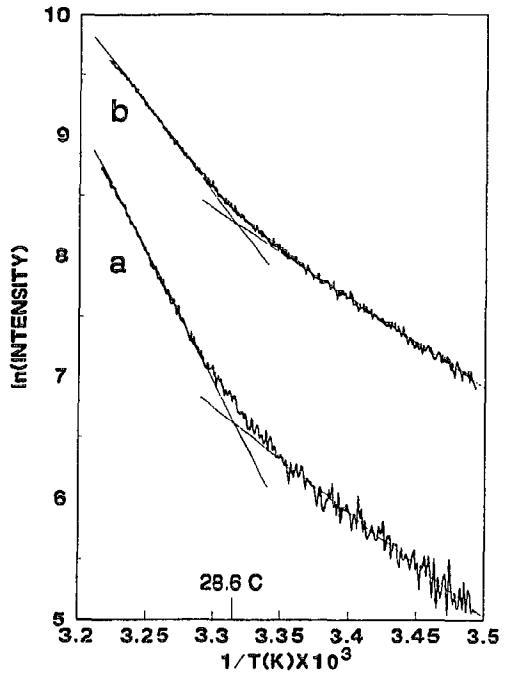

Fig. 1. Arrhenius plots of light emission intensity from (a) native and (b) ascorbate-driven lipid peroxidation.

Our measurements show that lipid peroxidation is sensitive to membrane lipid conformation. This relationship, until now unrecognized, links the important study of lipid peroxidation and its kinetics with membrane structure and dynamics. That such a link exists may suggest a more complex relation between peroxidative processes, membrane molecular structure and in vivo protective mechanisms [2] than previously envisioned.

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